Communications to the Editor

[Chem. Pharm. Bull. 31(6)2146—2149(1983)]

P.D. 1983
p. 2146-2149 (4)

ANTIFEEDANTS FOR THE LARVAE OF THE YELLOW BUTTERFLY, *EUREMA HECABE MANDARINA*, IN *LYCORIS RADIATA*

Atsushi Numata,*[,a] Tsuruko Takemura,[a] Hideyuki Ohbayashi,[a] Takako Katsuno,[a] Kyoko Yamamoto,[a] Kimihisa Sato,[b] and Shigeru Kobayashi[b]

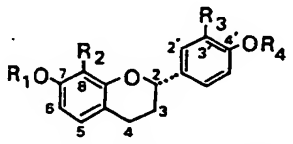
Osaka College of Pharmacy,[a] 2-10-65 Kawai Matsubara, Osaka 580, Japan and Faculty of Pharmaceutical Sciences, Tokushima University,[b] 78 Sho-machi-1-chome, Tokushima 770, Japan

In addition to the eight known compounds two new constituents were isolated as antifeedants for the larvae of the yellow butterfly, *Eurema hecabe mandarina* de l'Orza, from the methanol extract of *Lycoris radiata* Herb. Their structures were established to be (-)-3'-hydroxy-4'-methoxy-7-hydroxy-8-methylflavan (I) and O-methyllycorenine (III) on the basis of spectral evidence. Final evidence for the structure of I came from the synthesis of the racemate. Two of the known compounds, galanthamine and lycoramine, were isolated as carbonate. In addition to I and demethylhomolycorine (IV), lycoricidinol (VII) and lycoricidine (VIII), reported as plant growth inhibitors, were main antifeedants in *L. radiata*.

KEYWORDS——Amaryllidaceae; *Eurema hecabe mandarina*; alkaloid; flavan; antifeedant; *Lycoris radiata*

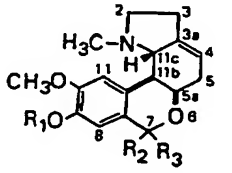
Our continuing search for insect antifeedants from plants[1] has led us to examine the active constituents from the bulbs of *Lycoris radiata* Herb.(Amaryllidaceae). Methanol extracts from the bulbs revealed potent antifeeding activity when tested against the 5th instar larvae of the yellow butterfly, *Eurema hecabe mandarina* de l'Orza, using the same bioassay as that reported previously.[1] Separation of active materials from the extract was made by several methods under monitoring by the antifeeding test. Feeding inhibitory activities were evaluated on the basis of the feeding ratio, which was calculated from the mean frass count of the 5 final instar larvae for test diets and control diets.

The methanol extract was fractionated into chloroform soluble and water soluble fractions. The former was purified by repeated silicic acid column chromatography to yield antifeeding active substances I, II and III. The latter was purified by a combination of LH-20 and silicic acid column chromatography



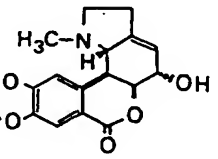
I: R_1=H, R_2= R_4=CH_3, R_3=OH

II: R_1=CH_3, R_2=R_3=R_4=H

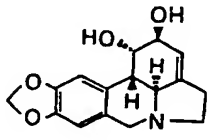


III: R_1=CH_3, R_2=H, R_3=OCH_3

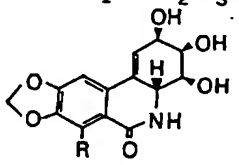
IV: R_1=H, R_2=R_3=O



V

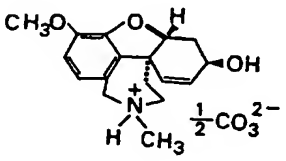


VI

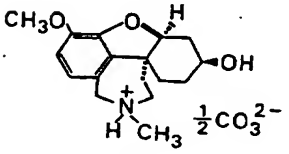


VII: R=OH

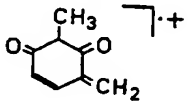
VIII: R=H



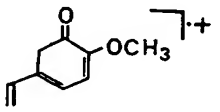
IX



X



A



B

and droplet countercurrent chromatography to give antifeeding active substances IV-X. Compounds IV, V, VI, VII and VIII were respectively identified by direct comparison with authentic samples or spectral comparison with the reported data[2] as demethylhomolycorine, hippeastrine, lycorine, lycoricidinol, and lycoricidine which had been already isolated from this plant. Compound II was also identified as 4'-hydroxy-7-methoxyflavan by comparison of the spectral data with those reported previously.[3]

Compound I, a colorless oil, $[\alpha]_D^{20}$ -31.0° (c=1.44, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 226 (sh)(4.21), 283 (3.73), 2.88 (sh)(3.71), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3530, 3340 (OH), 1600, 1510 (aromatic ring), was assigned the molecular formula $C_{17}H_{18}O_4$ on the basis of the MS (M^+: *m/z* 286) and the molecular formula of the acetate, $C_{21}H_{22}O_6$ (M^+: *m/z* 370.1406, Calcd: 370.1414). The ^1H NMR spectrum (DMSO-D_6, δ ppm) showed the presence of the five aromatic protons of 1,3,4- (6.56, 1H, d, *J*=13 Hz, 6.76, 1H, d, *J*= 13 Hz, and 6.83, 1H, br s), and 1,2,3,4-substituted benzene ring (6.33, 1H, d, *J*= 9 Hz and 6.87, 1H, d, *J*= 9 Hz), a methine proton attached to ether (4.93, 1H, dd, *J*= 9, 3 Hz), a methoxyl signal (3.77, 3H, s), two methylene protons (2.5-3.0, 2H, m and 1.8-2.2, 2H, m), a methyl group attached to benzene (1.98, 3H, s), and two phenolic hydroxyl protons (8.93, 2H, s). On addition of $MgCl_2$, the doublet at 6.33 and a singlet at 6.83 were shifted to downfield by about 0.16 ppm, showing that these protons are located in the *ortho*-position of phenolic hydroxyl groups.[4] Acetylation of I gave a diacetate

as an oily substance, which showed in the MS the fragments[5] corresponding to A and B at *m/z* 137 and 150 besides the molecular ion.

These observations show I to be (-)-3'-hydroxy-4'-methoxy-7-hydroxy-8-methylflavan. It is evident from the coupling constant of the methine proton attached to ether that a conformation in which the 2-aryl group is equatorial is strongly favoured.[6] The absolute configuration indicated in structure I is based on the previous observations that the S configuration is established for (-)-4'-hydroxy-7-methoxyflavan.[3] The natural product (I) showed identical spectral data to the racemate of I, synthesized by hydrogenation of the corresponding flavylium chloride. The flavylium salt was prepared according to the method of Cooke et al.[3] from 3-hydroxy-4-methoxyacetophenone and 2,4-dihydroxy-3-methylbenzaldehyde.

Compound III, mp 121-125°C, $C_{19}H_{25}NO_4$ (M^+: *m/z* 331), $[\alpha]_D^{24}$ +198.0° (c=1.04, MeOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1611, 1500, showed in the ^1H NMR spectrum ($CDCl_3$, δ ppm) the presence of N-methyl group (2.12, 3H, s), benzyl proton (3.16, 1H, m), aliphatic methoxyl group (3.55, 3H, s), two aromatic methoxyl groups (3.87, 3.89, each 3H, s), a methine proton attached to ether (4.31, 1H, m), a vinyl proton (5.50, 1H, m), a methine proton of a hemiacetal (5.52, 1H, s), two aromatic protons (6.79, 6.88, each 1H, s). On acid hydrolysis III gave lycorenine.[7] These facts show III to be O-methyllycorenine.

Compound IX, mp 218-222°C, $[\alpha]_D^{24}$ -98.8° (c=1.0, MeOH), was assigned the molecular formula $C_{17}H_{21}NO_3 \cdot 1/2\ H_2CO_3 \cdot H_2O$ on the basis of the elemental analysis and the following evidence. The IR spectrum (ν_{max}^{KBr} cm^{-1}) exhibited bands corre-

Table I. The Feeding Inhibitory Activities of Flavans and Alkaloids

Samples	Concentration %	Feeding ratio %	Samples	Concentration %	Feeding ratio[a] %
I	0.8	7.2	VI	0.4	34.0
	0.2	11.6		0.25	22.8
	0.05	35.8		0.1	39.5
II	0.8	20.8		0.05	53.5
	0.4	27.5	VII	0.25	13.0
	0.2	19.1		0.1	15.3
	0.05	66.5		0.025	29.3
III	0.8	32.4	VIII	0.25	21.9
IV	0.4	20.9		0.1	46.0
	0.2	20.0		0.025	44.2
	0.1	19.1	IX	0.4	21.9
	0.05	22.8		0.1	41.4
V	0.4	27.4		0.05	91.6
	0.2	23.7	X	0.8	40.5
	0.1	55.3		0.6	62.8

a) Strong feeding inhibitory activity, 0-20%; slight, 20-50%; none, 50% and greater.

sponding to N^+H (2650-2000) besides hydroxyl (3560, 3350) and benzenoid absorptions (1617, 1588, 1506). The ^1H NMR spectrum (CD_3OD, δ ppm) was almost identical with that of galanthamine, except that the signal of N-methyl group (2.98, 3H, s) appeared at lower field than that of galanthamine. The MS was in accord with that of galanthamine. These spectral data show that IX is the ammonium salt of galanthamine which can be easily converted into the original base by heating. Neutralization of IX with ammonia gave galanthamine, and carbonate anion was detected by the formation of calcium carbonate, which was confirmed by the evolution of carbon dioxide by treatment with hydrochloric acid. These observations show IX to be galanthamine carbonate. Compound X, mp 130-135°C (amorphous powder), $C_{17}H_{23}NO_3 \cdot 1/2\ H_2CO_3 \cdot 2\ H_2O$, was also identified as lycoramine carbonate from spectral properties and the result of neutralization.

The feeding inhibitory activities of I-X are shown in Table I. I, IV, VII, and VIII were main antifeedants in the bulbs of *L. radiata*. Since VII and IX show potent insecticidal action, besides antifeeding activity, against the larvae of the yellow butterfly, their antifeeding activities seem to be inferred by the poisoning action.[8] It is particularly interesting that VII and VIII, reported as plant growth inhibitors,[2a] show the feeding inhibitory activities against the insect.

ACKNOWLEDGEMENT The authors are very grateful to Prof. S. Matsunaga and Miss M. Kurimoto of this College for the MS measurements.

REFERENCES AND NOTES

1) A. Numata, K. Hokimoto, T. Takemura, and S. Fukui, Appl. Ent. Zool., 18, 129 (1983).
2) *a*) T. Okamoto, Y. Torii, and Y. Isogai, Chem. Pharm. Bull., 16, 1860 (1968); *b*) A. Mondon, and K. Krohn, Tetrahedron Lett., 1972, 2085.
3) R. G. Cooke and J. G. Down, Aust. J. Chem., 24, 1257 (1971).
4) H. Yamaguchi, A. Numata, E. Uemura, H. Kaneto, and A. Yokoyama, Chem. Pharm. Bull., 23, 1169 (1975).
5) *a*) H. Audier, Bull. Soc. Chim. Fr., 1966, 2892; *b*) A. J. Birch, and M. Salahuddin, Tetrahedron Lett., 1964, 2211.
6) J. W. Clark-Lewis, L. M. Jackman, and T. M. Spotswood, Aust. J. Chem., 17, 632 (1964).
7) T. Kitagawa, S. Uyeo, and N. Yokoyama, J. Chem. Soc., 1959, 3741.
8) R. F. Chapman, Bull. Ent. Res., 64, 339 (1974).

(Received April 21, 1983)